CLAIMS

1. A method for measuring free ligands in biological fluids in the presence of bound ligand and endogenous binding proteins, without disturbing the equilibrium between the free ligand and the protein-bound ligand, comprised of the following steps:

(a) incubating a sample of biological fluid with (i) a ligand analog tracer which, due to its chemical structure, does not bind to some of the endogenous binding proteins, (ii) a specific ligand binder and (iii) at least one specific chemical inhibitor reagent that inhibits the binding of the ligand analog tracer to other endogenous binding proteins;

(b) separating the ligand analog tracer bound to the specific binder from unbound tracer; and

(c) determining the concentration of free ligand in said biological fluid.

2. A method for measuring free ligands in biological fluids in the presence of bound ligand and endogenous binding proteins, without disturbing the equilibrium between the free ligand and the protein-bound ligand, comprised of the following steps:

(a) incubating a sample of biological fluid with (i) a ligand analog tracer which, due to its chemical structure, does not bind to some of the endogenous binding proteins, (ii) a specific ligand binder and (iii) specific chemical inhibitor reagents that alone or in combination inhibit the binding of the ligand analog tracer to other endogenous binding proteins;

(b) separating the ligand analog tracer bound to the specific binder from unbound tracer; and

(c) comparing the bound fraction in said sample to the bound fraction of a given set of known free ligand calibrators to determine the concentration of free ligand in said biological fluid.

3. The method of Claim 1 wherein the chemical inhibitor reagent is a substituted monoaryl organic compound.

4. The method of Claim 1 wherein the other endogenous binding protein includes albumin.

5. The method of Claim 1 wherein the chemical inhibitor agent is 2,4-dinitrophenol.

6. The method of Claim 1 wherein the chemical inhibitor agent is sodium salicylate.

7. The method of Claim 1 wherein the free ligand is a hormone, steroid, drug, drug metabolite, polypeptide, protein, vitamin, antigen or toxin.

8. The method of Claim 1 wherein the free ligand is a thyroid hormone.

9. The method of Claim 1 wherein the free ligand is a sex hormone.

10. The method of Claim 1 wherein the specific ligand binder is an antibody to said free ligand.

11. The method of Claim 1 wherein the specific ligand binder is immobilized on a solid substrate.

12. The method of Claim 1 wherein the specific ligand binder is carried on a polypropylene substrate.

13. The method of Claim 1 wherein the ligand analog tracer is N-^{125}I-L-triiodothyronine ~~succinimide~~ succinamide.

14. The method of Claim 1 wherein the ligand analog tracer is N-^{125}I-L-thyroxine ~~succinimide~~ succinamide.

15. The method of Claim 1 wherein the ligand analog tracer is labeled with a radioactive atom, an enzyme, fluorophor, light chromophore or chemiluminescent group.

16. The method of Claim 1 wherein the ligand analog tracer is labeled with at least one radioactive iodine atom.

17. The method of Claim 1 wherein the free ligand is testosterone.

18. The method of Calim 1 wherein the ligand analog tracer is iodinated 6-hydroxytestosterone-19-carboxymethyl ether histamine analog.

19. The method of Claim 2 wherein said known free ligand calibrators are prepared by adding different amount of ligand to ligand - free human serum, calibrated by equilibrium dialysis and assigned free ligand values.

20. The method of Claim 1 wherein said method is carried out at about 37°C.

21. The method of Claim 1 wherein said method is carried out at about pH 7.4.

22. The method of Claim 1 wherein the chemical inhibitor reagent is a dye.

23. The method of Claim 1 wherein the chemical inhibitor reagent is sulfobromophthalein.

24. The method of Claim 1 wherein the chemical inhibitor reagent is a fatty acid.

25. The method of Claim 1 wherein the chemical inhibitor reagent is oleic acid.

26. The method of Claim 1 wherein the chemical inhibitor reagent is a phenolic hydroxyl compound.

27. The method of Claim 1 wherein the chemical inhibitor reagent is an amino acid.

Ins B1 add C8 add G' add H'